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MUTATIONS IN THE GENUS NICOLAIERILLUS (B. TETANI)

STUDIES ON PATHOGENIC ANAEROBES VIII

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In a former paper,[1] it was suggested that in the study of anaerobic bacilli colony formation in deep agar was available for two purposes: for the differentiation of strains that behaved alike on ordinary mediums, and for the study of mutations. The objection will be made that if anaerobic colonies mutate, they are useless for systematic purposes. This objection holds good for the groups in which mutations occur readily and not for those in which they are rare. No other group studied in this laboratory shows such a wide range of colony types as does the genus Nicolaierillus[2] (B. tetani). The diversity of these colonies has been pictured in a preceding communication.[3] Mutations in deep liver agar are frequent in this proteolytic genus, while among nonproteolytic organisms, although mutations in colony form probably occur, they have not been noted. Moreover, in some genera it has been possible to correlate the different colony types with other characters that may be termed specific, while in the tetanus group it has thus far been impossible to do so. A study of a few mutations in the genus Nicolaierillus is herewith described. Apparently these organisms furnish a most excellent field for the close study of bacterial mutation—here only superficially investigated.

TECHNIC

The technic employed in this study has been described before.[1, 4] It is principally the ancient procedure of Liborius.[5] Inoculum was thoroly mixed in the first of three dilution tubes of deep agar. From this tube a suitable amount was removed with a Pasteur pipet to a second tube, this amount depending on the number of organisms in the original inoculum. The second tube was mixed and a larger amount (0.25-0.75) taken from it and placed in a third. Three tubes usually suffice to give a satisfactory picture of the flora

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[1] J. Infect. Dis., 1922, 30, p. 1.

[2] Heller, H. H.: J. Bacteriol., 1922, 7, Jan.

[3] J. Infect. Dis., 1922, 30, p. 18.

[4] J. Bacteriol., 1921, 6, p. 445.

[5] Ztschr. f. Hyg. u. Infectionskrankh., 1886, 1, p. 115.

of an inoculum. In case more chance for mutation is desired, two or three number 2 tubes may be inoculated. A third dilution tube should always be used to give a chance for obtaining in one tube a pure culture of the predominant biotype.

The shaft of agar from which inoculation is desired is blown from its tube into a Petri dish by means of a large-bore Pasteur pipet. The same pipet, with careful flaming, may be employed many times with no chance of contamination. The location of a desirable colony may be marked by the pipet groove in the agar and by a mark on the glass tube to indicate the distance of the colony from the end of the tube. Small colonies may be fished under the binocular or ordinary microscope but such procedure is seldom necessary for tetanus colonies. Fishing is accomplished with the pipet that is to be used for diluting the agar in the next three tubes. The glass is drawn to a needle point, broken and flamed, and inserted into the agar shaft to the desired colony, which is sucked up and transferred to the first dilution tube of a new series. Agar may be cut with a flamed (not a boiled) razor blade or knife; it is unnecessary to "sear" the agar, which process only melts it anyway.

THE HISTORY OF STRAIN T 087

The culture T 087 was received from Parke, Davis and Co. and is one of their regular toxin-producing strains. When first studied, its colonies in deep liver peptone agar were uniform, small, subspherical, with an irregular outline and no long woolly protuberances (plate I upper left figures). After some months of cultivation on meat medium the strain was thought to be contaminated with an organism forming woolly colonies. The small colony type was isolated but contamination by a woolly colony was again observed, and repeated shakings in agar dilutions showed that the supposed contamination must have been a mutation. The history of the behavior of this strain is here given in detail.

The original agar culture (Nov. 4, 1919) was inoculated onto meat and therefrom onto agar dilution tubes. One colony form was observed, the small type mentioned in the foregoing. A colony of this sort passed through a meat culture and was inoculated onto agar again. Two types of colonies were noted; a small one was fished onto meat. and this culture had had 5 passages on meat when, 9 months later, it was reinoculated onto agar, and showed 2 types of colonies in about equal numbers. The larger of these (TOL, plate 1) was replanted many times on agar of the same batch and no reversion or mutation was noted.

A small colony gave in dilution tubes both types, the small being most abundant. The third tube had 6 colonies of each type. The offspring of 2 large ones from this tube were all fluffy like TOL, with which they were probably identical. One small colony gave, in a series of 4 dilution tubes, only one woolly colony (TO 2), which may have been a contamination by TOL, but by its composition was probably a mutant. Its subsequent history was of interest. This one colony gave two types. In the second tube 58% were large. In the third tube 4, which were carelessly abandoned, were large, one was small, one smooth and larger. The story of the smooth larger colony on reinoculation onto agar tubes follows:

1. Very abundant colonies, 2 types.
2. Large, dense, and smooth, 390; small 96.
3. None.

Large dense smooth type one colony onto agar.

1. ?
2. Eighty-nine large, fluffy; 2 small.
3. Two fluffy (TOM, Plate 1).

The smooth large colony, intermediate type between TOM and TO 2 was, sad to say, not photographed, and it is a question whether it may have been the same as TOM or not.

A small colony from TO 2's first subculture, which represented the original type, gave in dilution tubes uniform small colonies of which a single one was markedly asymmetrical, having a woolly side and a smaller smooth side. Inoculation (fishing was done under the binocular) from the woolly half of this asymmetrical colony gave pure dense woolly colonies (TDW, plate 1). Inoculation from the smooth half gave in the third tube 40 dense woolly colonies and 44 smooth ones.

A smooth colony from a sister tube to that from which TO 2 was derived, a tube in which no woolly colonies existed, gave two types of colonies, 41% of them large. As I was at the time still under the impression that these large mutating colonies were all alike, this mutant was not photographed and was lost. Here is an instance, one of several in this series, in which a colony fished at random, had within it mutants that had caused no change in the shape of that colony. It is better to choose for the demonstration of mutation colonies in tubes that contain no mutant colony, so that the question of contamination by permeating growth is eliminated.

To return to the early tube in which there were 6 small colonies and 6 woolly ones: Another colony showed either contamination with TOL or mutation. Twenty-five % of the colonies were fluffy and the rest smooth One small colony from this generation gave 0.43% large colonies, another 2.9% large ones. These also probably represent contamination by the other type (TOL). One tube of this series contained 100 smooth colonies and no woolly ones at all. When one such colony was inoculated into agar tubes the second dilution tube showed a mutant, one colony in 250, radiate in structure, which type (TRW) in culture tubes did not resemble TOM so closely as it does in the photograph. The third tube contained only 2 small colonies, no woolly ones. One of these on inoculation into agar showed only one type (small) in the first tube, one type in the second (300 colonies), but in the third dilution tube were 12 small colonies and one woolly mutant (TLW), that was larger than all the others, which was easily isolated in the dilution series on which it was planted.

About as many transplants were made of smooth colonies that gave rise to no mutants as of those that did. Their history is omitted.

The photographs on the plate were made under identical conditions at the end of the experiment.

Three-day meat cultures of these 6 strains (T 087, TDW, TOL, TOM, TRW and TLW) were inoculated subcutaneously into guinea-pigs in 0.75 c c doses. The animals showed no symptoms of tetanus. Ten-day meat cultures gave a like result, and 7-day cultures in glucose-veal toxin broth containing blood inoculated on a third series of guinea-pigs caused no symptoms of tetanus. The behavior of all the T 087 strains was culturally entirely typical of that of tetanus bacilli[3] and strain T 087 had long been known as a standard toxin producing strain. Miss P. Schoenholz was so kind as to investigate the agglutination reactions of the organisms and found that all 6 substrains were agglutinated by serums made from strain TT (Tulloch's type 1) in titers from 1000-6000, strain TE (T 089 of the U. S. Public Health laboratory) in titers from 4000-20000 and strain T 087 (a serum that was made shortly after the arrival of the culture in this laboratory) from 4000-8000. It must be concluded that during the frequent fishing of the small type before the commencement of this experiment a wholly nontoxic mutant had been selected from among the toxin-producing colonies.

We have here a record of 4, or 5 or 6 different mutant types, all but one occurring during the 2 weeks' study of a tetanus strain. Apparently this strain mutates in so many directions that the classic demand that to prove bacterial mutation the mutant must be repeatedly found, cannot be satisfied without long search. Had the colonies not been photographed they would have been thought to be alike, and the requirement would have been technically, apparently, fulfilled. No contamination, either by other anaerobes than tetanus, or by aerobes, was noted during the whole study—and such contamination was feared and rather expected, for the laboratory is full of many kinds of anaerobes. Practically every unusual colony was fished and its behavior studied.

It was noted that if the colonies were fished when 24 hours old, mutants were hard to find, but if they were 48 hours old, in a large series of tubes they were usually to be found. Therefore, old material was investigated in order to find whether many mutant forms existed therein.

THE BEHAVIOR OF STRAIN T USA

This strain was furnished by Miss Muriel Robertson who, I believe, isolated it from a stock American strain by means of the Barber technic[6] during the tetanus investigation. She states that with it Tulloch made the serum with which his type 1 strains were identified.[7] It was received in the form of an egg broth culture in the fall of 1919 and bore the designation T USA II and the date September 6. Previously another culture of what was probably the identical strain had been received from Dr. McCoy of the United States Public Health Laboratory. This (TT) was labeled "Tulloch, Type I" and made large fluffy colonies without an opaque center and, more rarely, large opaque woolly colonies (Fig. 2, top row).

In March, 1920, the egg broth culture "T USA II" was inoculated onto meat and therefrom onto agar. Two types of colonies were observed. One was large and fluffy without an opaque center. The other was slightly smaller and was opaque. After isolation, these were photographed (Plate 2, second row), but already after only two successive transplants the original type of clear fluffy colony had been partly replaced by a colony with a small opaque center—T USA W. Both these types were typical tetanus bacilli and later formed high titer toxins that produced typical tetanus symptoms in mice during an immunity experiment.

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[6] Phil. J. Sc., B., 1914, 9, p. 307.

[7] J. Roy. Army Med. Corps, 1917, 29, p. 631; Proc. Roy. Soc., B., 1919, 90, p. 145.

In October, 1920, a meat culture was again made from the original egg broth tube. On agar its colonies were of only one type, large and fluffy. A sister meat culture was sent to Dr. Ivan C. Hall, who complained that it contained two kinds of tetanus colonies, an opaque one that behaved normally and a fluffy one that could not be purified, the opaque type continually reappearing therein.

In April, 1921, the meat culture made in October, 1920, was planted directly into new agar dilution tubes. Instead of one single fluffy type, such as was found in such a transplant in October, a galaxy of divergent types appeared. From the second dilution tube a number of these were fished into a new series of agar tubes and were isolated in pure culture (Plate 2, large group, all but T USA LC). Some of these were alike but between others of the strains were marked differences. The original fluffy type was, apparently, lost. Certain of the colonies were similar when observed with a hand lens, but differences could be observed between them when they were photographed. Such were T USA M and P. Others can be correlated by photographic procedure (USA X and P). T USA F is a larger form than the original type. T USA LC, the only strain pictured that was not isolated from the second dilution tube from which the others originated, is an independent derivative from the original stock, which was a fluffy clear centered strain, and it resembles F in contour but not in size. T USA O was consistently larger than T USA X and P, which are probably the same sort of mutant as the original T USA S. T USA B is distinctly smaller and more smoothly contoured than any T USA S-like colony observed. And, finally, because the parent tube had lain for some days before it was fished, a successful subculture was obtained of a very minute form, T USA T, which is hardly to be discerned with a hand lens after 24 hours' growth, but forms small spherical colonies when 48 hours old.

Thus we see that a tetanus strain, left long on a medium, like egg, that furnishes little nutriment, retains its type, probably through failure to multiply. But, if it be sown on a well-buffered highly nutrient medium, like meat, it will continue to multiply, in so doing it may mutate, and the mutant forms complete, some dying out, some sporulating and waiting for a better day, others increasing, till after a few months, if mutation be active, the culture tube may contain a multitude of biotypes, some better able to multiply on another substratum like agar, and some less able to multiply on it, and some multiplying as abundantly as the parent type but taking, in colonies, different forms.

These strains—T USA-F, M, LC, O, X, P, and T—were all typical tetanus bacilli; 3-day old meat cultures of them when inoculated subcutaneously into guinea-pigs in doses of 0.75 c c killed the animals with symptoms of tetanus inside of 48 hours.

THE HISTORY OF THE STRAIN TG

This strain was isolated from gelatine at the United States Public Health Laboratory from which I received it in an agar stab in November, 1918. It was planted onto meat on November 6, therefrom onto meat June 25, 1919. July 2, agar shakes from this meat culture gave colonies that were invisible in 24 hours, and are shown at that age magnified 50 diameters in the photographs (Plate 3, top left). Colonies incubated longer than 24 hours grew apace and when 5 days old were as in the series below. A smooth colony was fished from this tube and after passage on meat gave colonies in agar that were denominated "irregular, smooth and minute." On July 14, the meat culture that was planted June 25 was again inoculated onto agar tubes and the result was small irregular colonies, at 24 hours indistinguishable, at 48 hours definitely of two sorts—lenticular and woolly. Subsequent transplants were made from the stock termed "irregular, smooth and minute" in September, and the first agar shake showed small irregular compound lenticular colonies (Plate 3, upper right) that, when 3 days old, were not to be distinguished from one another, but when 5 days old were of 2 types, some loose and woolly, some smooth and lenticular, and 2 with bushy protuberances. Both types were fished, but, sad to say, the smooth type was lost and has never been recovered. The strain now is a woolly one (the 4 colonies below) with an enormous range in colony size that is exceedingly baffling to work with. Three quarters c c of 3-day old meat culture inoculated subcutaneously into a guinea-pig caused the death of the animal in a few hours.

It must be borne in mind that this work was done before the significance of these mutations was comprehended, in order to be sure the strain was pure, and in order to take photographs. Whether the differences seen between the two top groups of figures on plate 3 represents a mutation it is now impossible to say. It is probable that it does. But that the woolly type, now the only one preserved, is a mutation from the smaller type cannot be doubted. Of interest is the fact that when crowded the colonies resemble closely their ancestral form. In crowded tubes these compound lenticular colonies are permanent and do not turn woolly except when the agar has not been thoroughly mixed and there is abundant nutriment for the colonies at the edge of the culture. But no pure lenticular form can be isolated from them, for as soon as they are planted in sufficient dilution lenticular forms are almost impossible to find, practically all the colonies being woolly. These woolly colonies vary greatly in size, but whatever sized colony may be chosen for inoculation into dilution tubes the result is always the same—the colonies vary from minute ones to large fluffs with a clear zone about the center.

STRAIN TL

This was received from Dr. S. H. Gilliland and was apparently a pure biotype that made a transparent colony with a tendency to form a thickened cortical layer (Plate 5, top left). Two years later a meat culture of the strain showed heavy opaque colonies of about the same size as the older ones (TLC, second row, left). Search revealed a few transparent colonies among them. After 2 transplants, the transparent type (TL B, top row, right) was recovered and it was impossible to demonstrate whether or not it was identical with the original type. Both of these strains (TLC and TLB) were highly pathogenic for guinea-pigs, causing marked asymmetry and rigidity of the body followed by the death of the animals inside of 48 hours.

It is of interest that these colonies showed a very small range of variation in size. The strain TG in its woolly form shows similar colonies that are a little more irregular in contour than those of TL, but they have a very wide range of variation in size. Strain TP ("Smith, from Pease" sent me by Dr. G. W. McCoy) forms more opaque colonies with a great range in size, utterly unsuited to the study of mutations.

STRAIN TVP

Strain TVP was isolated from an ivory vaccine point by Miss Bengtson and sent to me by Dr. G. W. McCoy. It forms the largest colonies in the whole collection, which is of interest from an epidemiologic point of view when one considers its origin. On first transplantation 2 kinds of colonies were noted. A loose fluffy type was isolated, and gave rise to colonies somewhat inconsistent in behavior, shown in Fig. 4, top and center. Two years later, 2 types, TVPF and TVPD which is derived from TVPF, were discovered in the original tube. What relationships these bear to the colonies pictured earlier is not clear. Three-day meat cultures of TVPF and TVPD killed guinea-pigs in doses of 0.75 c c with symptoms of tetanus in 24 and 48 hours, respectively.

STRAIN T McC

T McC was a culture given by Dr. A. T. MacConkey to Miss Robertson who gave it to me in 1916. The agar colonies depicted in the first photographs (plate 5, second row, center) were derived from a single colony in agar. The second photographs (second row, right) were later derived from the original culture without intermediate fishing, the batch of medium was a different one, but the conditions were the same. These photographs represent, in all probability, different biotypes, and the opaque form may have been derived from the other.

STRAIN T 220

This was received in meat culture, in 1918, from Miss Robertson who isolated it from a wound. With it Tulloch[7] made the agglutinating serum that enabled him to establish his type 3. It apparently existed in 2 forms in the meat culture as it was received, for transplants shortly from that culture have twice shown 2 forms (plate 5, third row). These have been worked with quite extensively and no other type has been noted in the cultures. It is not known which form gives rise to the other. Strong toxins that produced typical tetanus in mice were produced by both strains.

STRAIN VT

This was given me by Dr. K. F. Meyer, who secured it from a vaccine process in a fatal case of tetanus in a little boy who probably became infected through playing in a stable. When first studied, 2 very different types of colonies were noted that, when isolated, were photographed (plate 6, first and second rows). Strong toxins that produced typical tetanus in mice were formed by both strains.

THEORETICAL CONSIDERATIONS

Tetanus strains mutate readily in protein mediums in which they are able to multiply actively. This mutation is apparently much more frequent than in the case of nonproteolytic anaerobes. The phenomenon is in accordance with the hypothesis,[2] that proteolytic anaerobes are the products of a more far-reaching evolution than nonproteolytic ones. That is, the proteolytic anaerobes always have mutated more frequently on protein mediums than the others, and that is why they have attained their specialization.

Some strains of tetanus bacilli apparently mutate more readily than others.

The mutations of these organisms, frequent and striking as they are, never, so far as I have been able to observe, result in anything but the formation of typical tetanus bacilli. The derivation of forms differing from their ancestors in several important characters should be considered as possible only after the passing of a far longer period than that noted by certain workers with anaerobes (Grassberger and Schattenfroh;[8] Kolle, Ritz and Schlossberger[9]). A worker claiming to change a blackleg bacillus into a Welch bacillus would, in my opinion, in order to substantiate his claims, have to show pure cultures of several intermediate biotypes, for apparently mutation of several important characters does not occur, either at once, or in rapid succession. It is improbable that radical changes involving many characters of an anaerobe occur in short spaces of geologic time; otherwise we would not find universal common types all over the world.

As in the case of higher organisms, mutation may be either favorable or unfavorable to the existence of the organism. Probably numerous mutations affect the metabolism neither favorably nor unfavorably. The chances of detecting a favorable mutation are far greater than are those of detecting an unfavorable one. This does not correspond with the findings that Beijerinck[10] and others have made with aerobes;

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[8] Arch. f. Hyg., 1901, 48, p. 50; p. 1312; 1905, 53, p. 58.

[9] Med. Klin., 1918, 14, p. 281; München. med. Wchnschr., 1919, 66, p. 348.

[10] Folia mikrobiologia, 1912, 1.

Beijerinck believes that acquisition of characters by mutation has not been demonstrated for aerobic organisms. This is doubtless a matter of interpretation, but it is far easier to demonstrate the acquisition of a power by an anaerobe than the loss of one, when deep colony methods are used. The process of identifying mutations in the study of strain TO87 depended frequently on the detection of the mutation in the very colony in which it occurred—one among perhaps 100 or 500 others. This was easy because such colonies are bigger or fluffier than their neighbors, or they are asymmetrical. The chances of deliberately fishing a mixed colony in which a less capable mutant exists are remote—unless, indeed, there should appear an inhibitive or autolytic action like that noted for the colon bacillus by Bordet and Ciuca.[11] But in old cultures such less capable forms may exist and may be discovered by careful search. In the process of this investigation many minute colonies were fished before any were found that bred true. Colonies descended from spores and colonies near the wall of the tube may be smaller than their neighbors, but are usually of the same biotype.

No case of reversion has, in this brief study, been discovered. Beijerinck terms the reappearance of pigment forming prodigiosus colonies in nonpigment-producing strains "reversion." Pigment is a substance that may be analyzed and such reversions may thus be definitely proved. But when a mere matter of colony form is under consideration, it will not be wise to call the reappearance of an ancestral form in a pure mutant culture a reversion, unless we know something of the chemical nature of the processes involved.

According to Dr. Thomas Morgan (pers. com.), there are two kinds of possibilities that may determine reversion in bacteria, a recombination of two genes to produce a remote ancestral type being probably restricted to forms in which amphimixis occurs. The reversions which we should consider may depend on the tautomeric action of a gene that is always present and that may balance one way or another, or they may depend on an entirely new mutation that produces the same character as was possessed by an ancestral type. In the first case, the reversion will occur repeatedly in a certain percentage of cases; in the second case it will be exceedingly rare.

The study of mutating colonies will enable the systematist to deduce which metabolic characters are least constant by making special mediums containing substances that are to be investigated, and studying the colony formation in deep agar shakes made of such medium. This method is analogous to the well-known one of putting indicator in special sugar mediums for use with aerobes. If an indicator could be found that would not be reduced in the presence of anaerobic growth, it would be of great value in determining anaerobic mutations on carbohydrates. Such substances as furnish material on which the bacteria readily mutate, may be rigorously excluded from a standard medium on which the habits of various strains may be compared. An easy way is thus offered of avoiding expensive and time consuming tests of the behavior of certain organisms when such tests should never be used for systematic purposes on account of the mutation of the organisms under the conditions afforded by those tests.

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[11] Compt. rend. Soc. de biol., 1920, 83, p. 1293.

The significance of these mutations to the biochemist is great. His road is a difficult one to plan. He must make quantitative determinations and yet know that they may be impossible to confirm. Specific chemical action exists, surely, but it will be difficult to determine what is specific and what is not. The chemical behavior of anaerobes should be studied for several strains of the same group that differ in every known way.

Mutations are also of importance to the therapeutist. DeKruif[12] has recently found two types of the bacillus of rabbit septicemia living side by side, both virulent for rabbits, one far more so than the other. For organisms whose pathogenicity is dependent on their invasive power, such mutations are of great significance for purposes of immunization. Virulence depends very much on the ability of the organism to multiply in its environment. It has been customary of late for surgeons to depend, in certain types of tetanus cases, on antitoxin to help the body to fight off an infection that would otherwise remain somewhere near equilibrium, instead of operating to sterilize the seat of the infection. Theoretical considerations are radically against such procedure. Any day a new form of tetanus bacillus may arise that is four or five times as well able to multiply as were its ancestors, and this type may upset the equilibrium. Such a phenomenon may explain many cases of latent tetanus.

No effect of these observed colony mutations on toxin production has been noted, although it is reasonable to presume that by multiplying more extensively a strain will produce a stronger toxin. Filtered toxins prepared from some of the mutant strains had similar titers. All strains tested produced strong toxins in 3-day meat cultures except T 087 and its derivatives which behaved consistently with one another.

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[12] Jour. Am. Med. Assn., 1921, 76, p. 651.

Miss McRoberts of this laboratory is investigating the agglutination reactions of these strains and will report on them separately. One is forced to presume that these nutritional mutations are more frequent and superficial than such as would be discovered by immune reactions where a large protein fraction of the bacterium or a secreted toxin is the substance estimated.

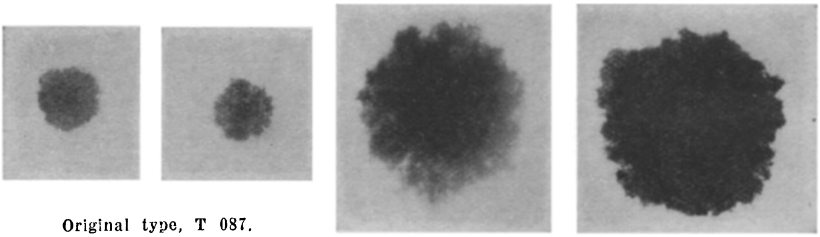
TECHNICAL DEDUCTIONS

In choosing anaerobic material for the study of mutations one may seek in the literature or in his own notes for evidences of irregularity in the behavior of certain types of organisms. The worker should first investigate whether the irregularities are due to contamination, for the literature is permeated with reports based on the behavior of contaminated cultures. If pure cultures have been found that behave peculiarly, a medium may be chosen that will emphasize the peculiarity. Deep colonies in this medium should be craefully observed, and, if the work is undertaken seriously, the colonies should be occasionally photographed. A species of organism should be chosen that does not form much gas or grow readily through agar mediums as though they were broth. Strains selected for particular observation should make symmetrical colonies that show little variation. A strain that normally is lenticular but occasionally sends out woolly processes, such a character being quite within the limits of variation for the biotype, is not well adapted for the study of mutation.

One should use the same batch of medium throughout a given experiment whenever possible (plate 5, lower series).

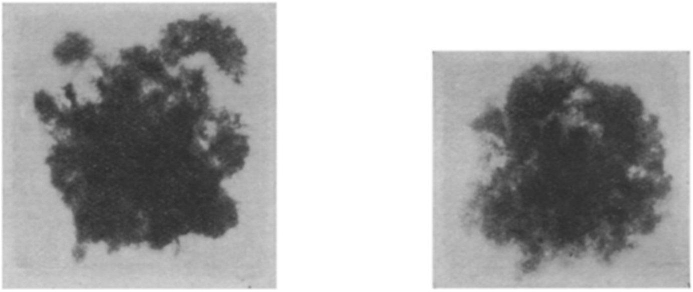
A type of photographic plate should be used that covers a wide range of lighting conditions and can always be procured. Detail, in the photography of colonies, is more important than contrast.

One should refuse to undertake to study mutations in a dusty room where there are draughts or people pass frequently—otherwise other types of anaerobes will probably make their appearance in the culture.

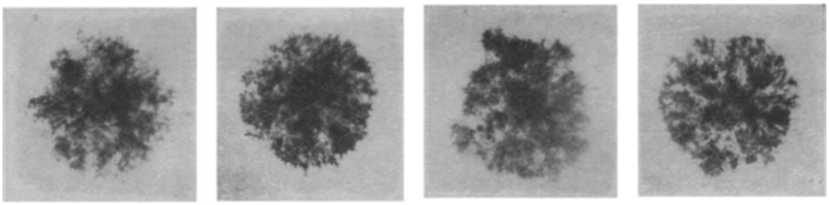


Original type, T 087.

TDW.

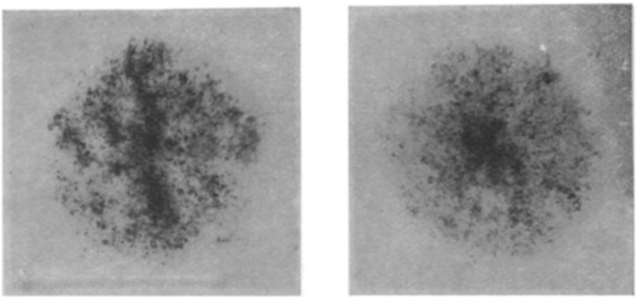


TOL.



TOM.

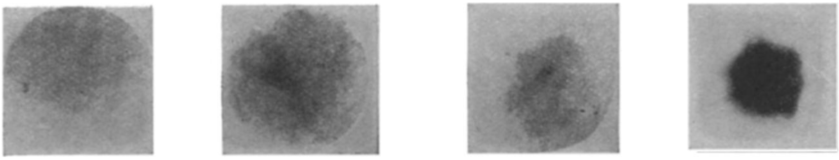
TRW.



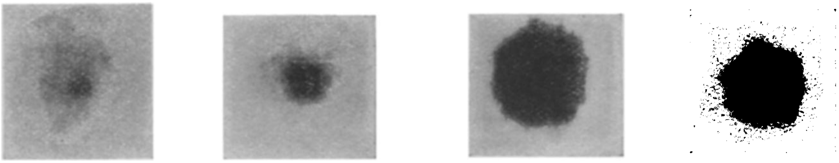
TLW.

Fig. 1.—The mutations of strain T 087. Five pairs of photographs show what are probably 4 types of mutants derived from the minute type in the upper left hand corner. They arose during two weeks' study of the behavior of this strain on deep liver peptone agar. All were grown simultaneously for 24 hours on the same batch of medium under identical conditions; colonies few; × 50.

Unless otherwise indicated this and the following illustrations represent colonies that were incubated for 24 hours at 37 C. in deep liver peptone agar and were enlarged 50 diameters.



Strain TT, probably identical with strain T USA; colonies not numerous, 1919.



Strain
T USA W.

Colonies not numerous, 1919.

Strain
T USA S.

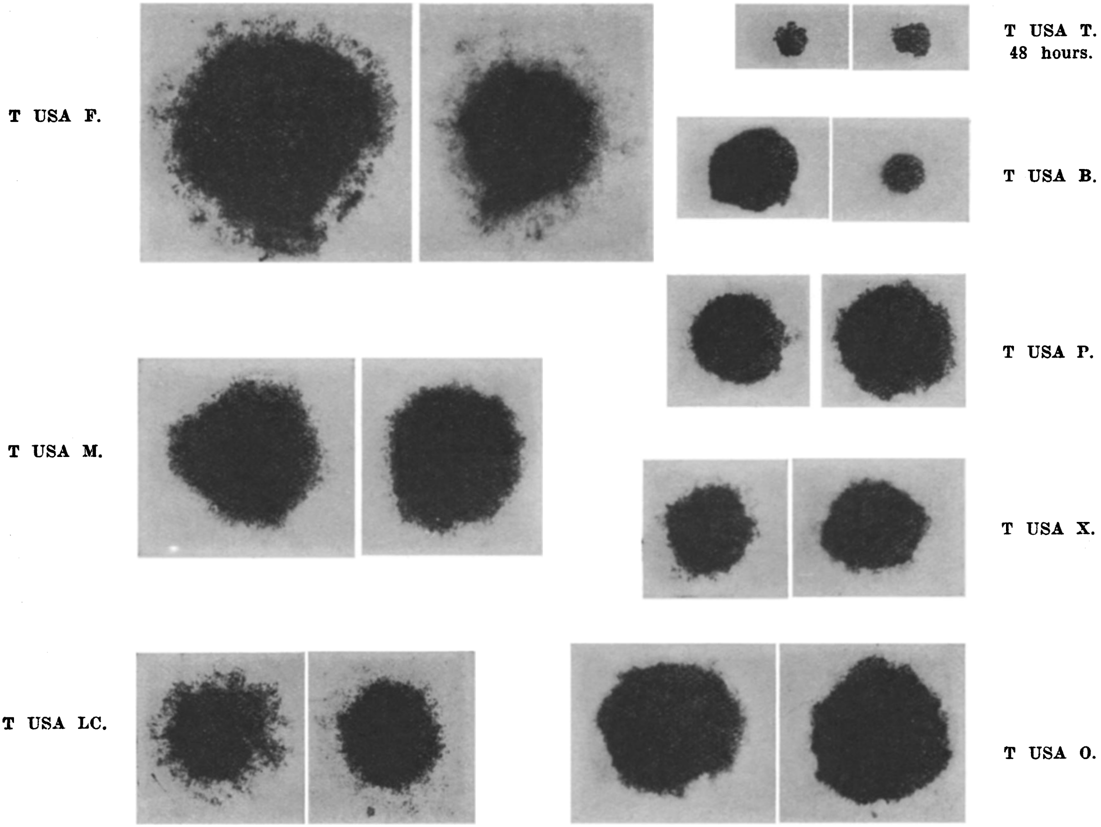
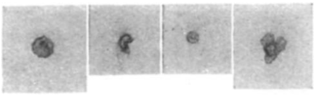
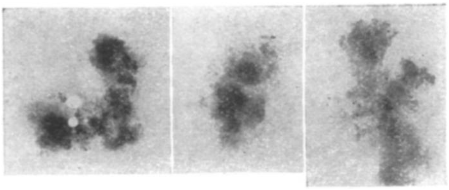


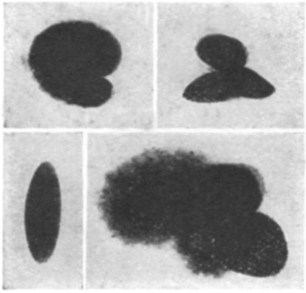
Fig. 2.—Mutations of strain T USA. In October, 1920, a meat culture of T USA (W) showed the fluffy type only. In March, 1921, the same meat medium tube that had long stood at room temperature showed a number of biotypes. The original form was apparently lost; some new types were larger than the original; all were denser and two smaller. The conditions of growth for the members of each of the three series were identical. The lower series were all taken on a Seed No. 23 plate; colonies few; × 15. The upper series were taken on softer plates at 50 diameters and were reduced to 15 diameters.



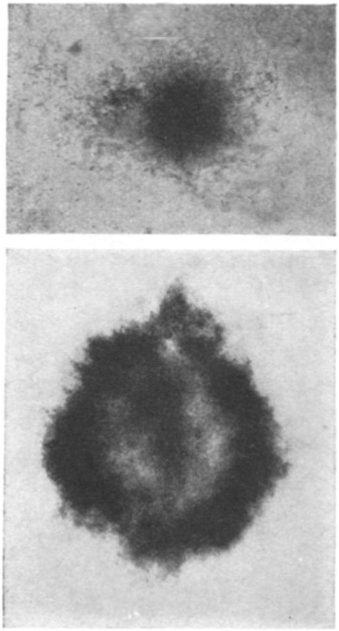
July 3, 1919; colonies moderately abundant; × 50

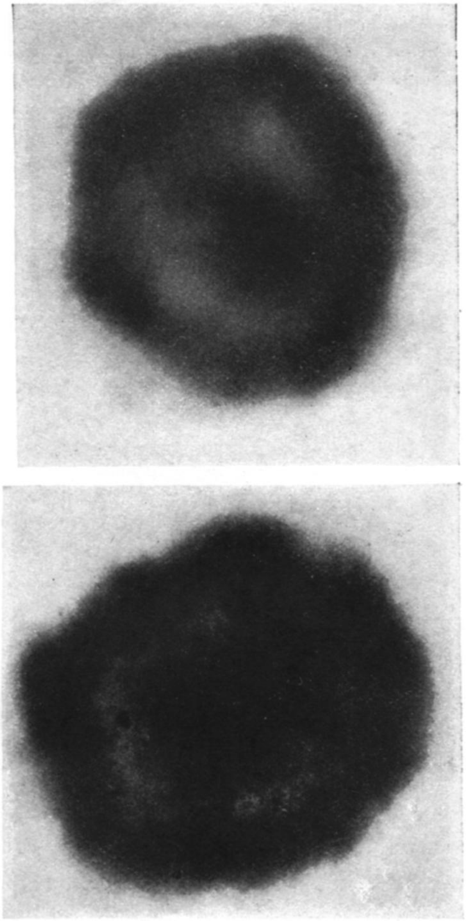


Oct. 2, 1919; colonies few.



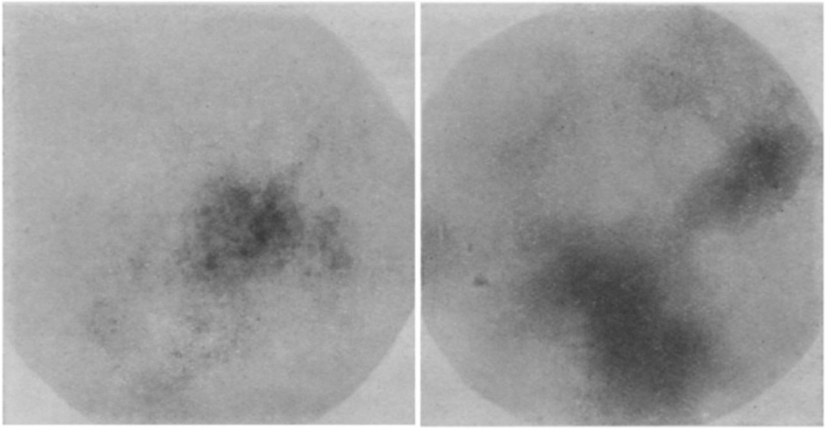
The above type, 5 days at 37 C.; colonies few; × 15.



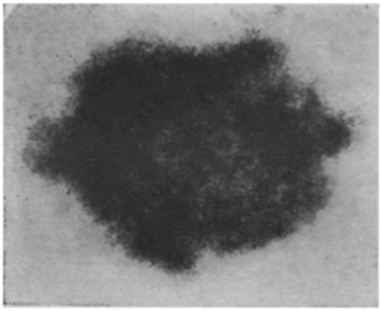


March, 1921, colonies not numerous.

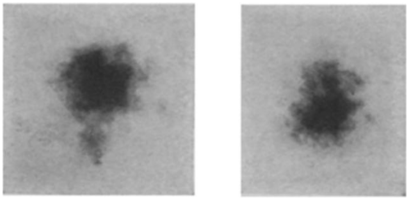
Fig. 3.—Changes in the behavior of strain TG. The origin of the woolly form from that of Oct. 2, 1919, was observed. This is a type with great range in size of colony.



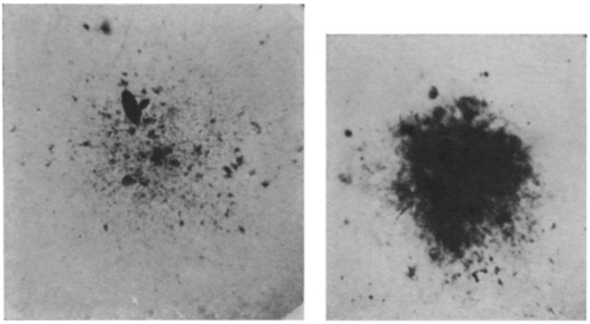
Oct. 28, 1919. Colonies few. December, 1919.

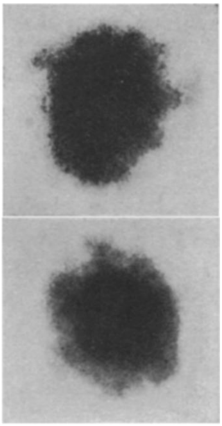


Oct. 22 ; colonies few ; × 15.



Oct. 28, 1919 ; colonies few ; × 15.

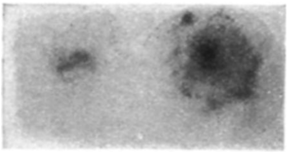




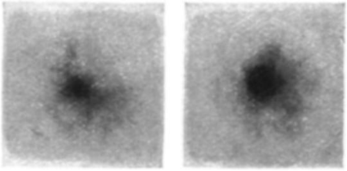
Strain TVP F. Strain TVP D.

Colonies few ; × 15.

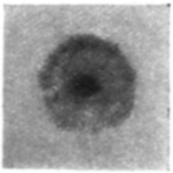
Fig. 4.—The behavior of strain TVP. The upper 5 photographs show the inconsistent behavior of the strain. The lower ones, taken under identical conditions, show 2 types isolated from the original material in April, 1921.



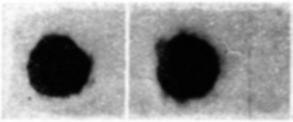
Colonies few; strain TL,
December, 1919.



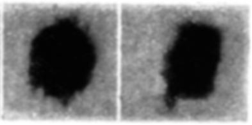
Colonies not numerous.
Strain TLB, April, 1921.



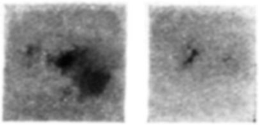
Sole colony.



Strain TLC, April, 1921;
colonies few.

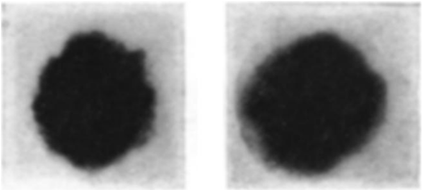


July 7, 1919.

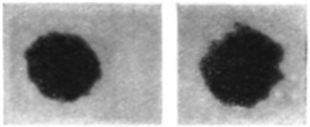


Sept. 30, 1919.

Strain TMcC.



Strain T 220 W.



Strain T 220 S.

Colonies not numerous.

Pairs of types noted at various times.

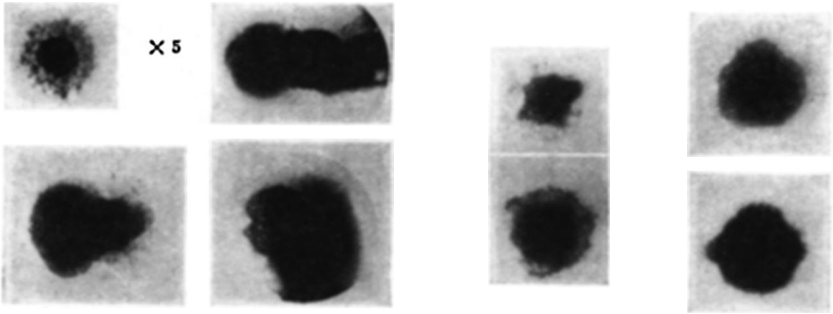
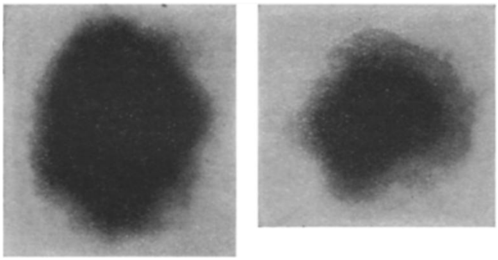
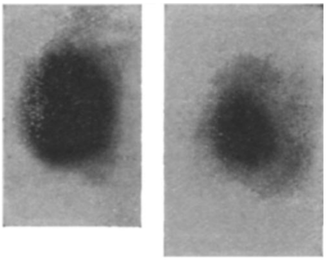


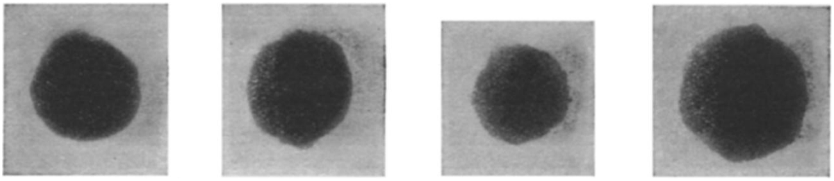
Fig. 5.—Simulated mutation strain T 220 S. This strain forms in 5 days colonies like the first (× 5) of this series. Cultures from the center gave dense woolly colonies (on the right) on stiff agar; cultures from the edge gave compound lenticular ones (the 3 flat colonies on the left) on soft agar. Subsequent transplants on the same batch of medium showed that the strains were identical. The two middle forms are from an intermediate medium. Colonies few: 48 hours old; × 15.



Strain VTW; colonies few.



Colonies moderately abundant.



Strain VTS; colonies few.

Strain VT gave the two types when first observed in meat culture.

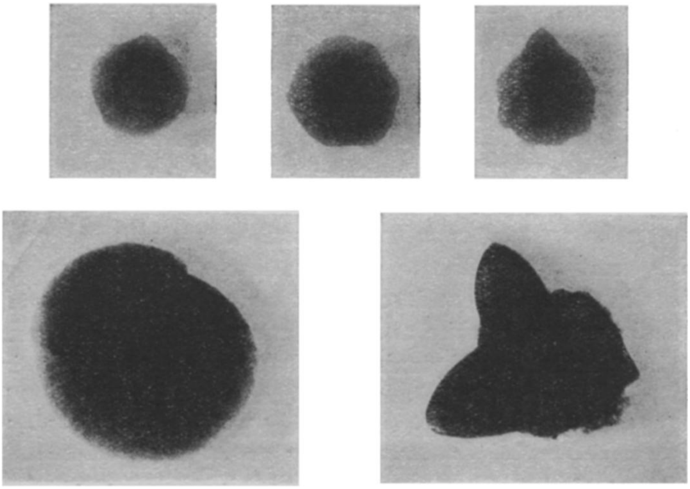


Fig. 6.—Strain TA: the upper colonies were taken in June, 1919, the lower ones in April, 1921, under approximately identical conditions; colonies few; × 50. It is obviously impossible to determine from these data whether mutation occurred or not.